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应用 RAPD 技术研究阿尔卑斯山黄花茅居群内的遗传分化

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Subpopulation differentiation of *Anthoxanthum alpinum* (Poaceae) along an altitudinal gradient detected by random amplified polymorphic DNA

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Abstract Random amplified polymorphic DNA (RAPD) phenotypes generated by 15 primers were scored in 5 subpopulations of *Anthoxanthum alpinum* along an altitudinal gradient. Although few subpopulation-specific markers were found, a significant population differentiation was revealed by the use of principal factor analysis and Ward's cluster analysis. The result was also confirmed by the significant positive correlation when comparing both the matrix of similarity between the individuals within subpopulations and the matrix among subpopulations, as well as the matrix of distances and the differences of altitude between the subpopulations (Mantel test). This study suggests that the differences of altitude between subpopulation sites may result in the differences of phenological period for flowering and growth which restrict gene flow.

Key words *Anthoxanthum alpinum*; Genetic differentiation; RAPD; Altitudinal gradient

Genetic differentiation of natural populations is considered as a dynamic process depending on the balances between gene flow and selection. Gene flow acts as a unifying factor. A substantial gene flow can hamper genetic differentiation. Selection which favors adapted genotypes in a heterozygeneous environments can override the homogenizing forces of gene flow, even when gene flow occurs (Caisse & Antonovics, 1978; Endler, 1977; Antonovics *et al.*, 1971).

Altitudinal gradients have been favoured for the study of ecotypic differentiation for a long time (Oyama *et al.*, 1993; Clausen *et al.*, 1940). The advantage of elevational transects is that rapid environmental changes often occur over short distance, e.g. the temperature decreases and the precipitation increases generally with the increase of altitude (Ozenda, 1985). Moreover, the differences of altitude could result in the changes in flowering onset and the period for growth, thus restricting gene flow between subpopulations of different altitudes (Galen & Kevan, 1980; Waser, 1978).

Recently, random amplified polymorphic DNA (RAPD) markers have become increasingly popular in their applications to population biology in spite of their properties of dominance. The advantages of this technique over conventional molecular markers such as RFLP are that large numbers of samples can be analyzed economically and quickly, that only minimal-quantites of material is

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needed for each sample, that the specific DNA fingerprints obtained are independent of ontogenic expression, and that most of the genomes can be sampled with a potentially unlimited number of markers. RAPD markers have been used to identify different individual genotypes and species (Liu & Furnier, 1993; Halward *et al.*, 1991), to study mating system in natural populations (Arnold *et al.*, 1991), to assess patterns of paternity and kinship (Lewis & Snow, 1992), and to detect and analyse genetic variation (Castagnone-Sereno *et al.*, 1994; Dawson *et al.*, 1993; Williams *et al.*, 1990).

In this study, we report the use of RAPD markers to assess genetic differentiation between the subpopulations of *A. alpinum* along an altitudinal gradient.

1 Materials and methods

1.1 Plant materials

Anthoxanthum alpinum Löve & Löve (Poaceae) is a perennial grass. Individuals of this species are diploid, predominantly outcrossing (Felber, unpublished data; Zeroual-Humbert-Droz, 1995). In the Alps, *A. alpinum* covers a considerable altitude range because it grows throughout the subalpine and alpine zones above 1700 m (Felber, 1986; Favarger, 1962). At lower elevations, *A. alpinum* is replaced by a closely related species, *A. odoratum* (Felber, 1988, 1986).

Sampling was performed at Belalp of the Swiss Alps. It is located in the southern part of the Aar massif of the Aletsch region on the right side of the Rhone valley. An altitudinal gradient was set up on an eastern slope, located on the versant of the Hofathorn between 2020 m and 2830 m with a distance of 1.75 km.

Subpopulations were sampled every 200 m altitude intervals. In 1993, four subpopulations were collected and sampling was completed in 1994 with an additional subpopulation (2830 m) in order to cover all altitudes. Each subpopulation consisted of 50 ~ 80 individuals collected at a distance of at least one-meter. The collected plants were then cultivated in the experimental garden of the University of Neuchatel.

1.2 DNA isolation and polymerase chain reaction (PCR)

Experiments were conducted in 1995. Five individuals were randomly chosen for each subpopulation. Total genomic DNA was isolated from fresh leaf material of single plant growing in the experimental garden by using the standard CTAB protocol (Doyle & Doyle, 1987). PCR was performed in volumes of 25 μl consisting of about 10 ng of genomic DNA, 1.5 μM primer (Operon Technologies Primer OPB and OPP series: no. 1 ~ 20.), 1 × Goldstar (polymerase) reaction buffer, 1.5 mM MgCl, 2.5 mM each of dATP, dCTP, dGTP and dTTP, 0.625 unit of Goldstar DNA polymerase. The thermal cycle was set for 1 cycle at 94℃ for 3 min. followed by 35 cycles of 30 sec. at 94℃, 45 sec. at 36℃ and 1 min. and 30 sec. at 72℃. and completed by one cycle of 7 min. at 72℃. Five μl reaction mix of each sample was loaded and run on minigel (1.6% agarose in TBE buffer containing ethidium bromide) and visualized under UV-light.

1.3 Data analysis

$$d_{jk} = \left[\sum_{i=1}^{P}(x_{ij} - x_{ik})^2\right]^{\frac{1}{2}}$$

Ward's cluster analysis and principal factor analysis, by the use of computer program STATISTA, were made to get an overview of the genetic variation and differentiation in the materials studied. Euclidean distance in the cluster analysis is defined as a measure of variance between two entities, j and k. In the principal factor analysis, factor 1 (axis 1) described 35% of variation, factor 2 (axis 2) decribed 10% of variation, and factor 3 (axis 3) described 7% of variation. In order to estimate the level of significance of genetic differentiation between the subpopulations and the correlation between the genetic differentiation and geographical distance (altitudinal gradients therein), we tested the correlation between matrix of similarity of individuals within subpopulations and com-

pared the matrix of distances between the paired subpopulations with the differences of altitude by a Mantel test. 999 permutations were performed.

2 Results

Forty primers of OPB and OPP series were used for an initial screening. Finally, fifteen of the OPB series, which generated informative amplification products, were chosen for the whole experiment. The information of RAPD primers used in the analysis of genetic variation in this study, the mean number of products per subpopulation and the number of unique products are presented in Table 1.

Table 1 RAPD primers used in the analysis of variation in 5 subpopulations of *A. alpinum* along an altitudinal gradient.

Primer code	Sequence (5′ to 3′)	Mean number of products per subpopulation					No. unique bands
		2020	2200	2405	2575	2830	
OPB-01	GTTTCGCTCC	6.8	4.8	8.0	8.0	10.0	4
OPB-02	TGATCCCTGG	5.2	5.8	4.4	4.6	6.4	0
OPB-03	CATCCCCCTG	5.4	6.6	4.8	5.2	5.6	0
OPB-04	GGACTGGAGT	6.0	5.4	3.4	4.0	4.6	1
OPB-05	TGCGCCCTTC	9.2	10.8	9.2	7.8	10.2	0
OPB-07	GGTGACGCAG	10.6	10.0	7.4	10.0	10.2	1
OPB-08	GTCCACACGG	4.6	4.4	4.6	4.2	5.6	1
OPB-09	TGGGGGACTC	4.8	5.4	5.2	5.8	4.4	1
OPB-10	CTGCTGGGAC	4.6	3.6	4.4	4.0	4.2	1
OPB-11	GTAGACCCGT	5.8	6.4	5.6	4.8	5.6	0
OPB-12	CCTTGACGCA	5.8	6.4	4.8	5.8	6.6	0
OPB-13	TTCCCCCGCT	5.4	4.8	4.8	4.2	4.2	0
OPB-14	TCCGCTCTGG	3.4	3.2	3.6	4.0	3.6	0
OPB-15	GGACGGTGTT	4.0	4.0	5.2	5.0	5.4	0
OPB-16	TTTGCCCGGA	3.4	3.0	3.6	3.6	4.4	0
Mean		5.7	5.6	5.3	5.4	6.1	

Considerable variation was found within subpopulations in this study, and only few subpopulation-specific RAPD markers from 15 variable oligonucleotide primers were identified for 25 individuals from 5 subpopulations (Table 1). However, a tree diagram from Ward's analysis of cluster showed that 25 individuals were clearly divided into three larger groups while linkage distance was 7.6. In the diagram, the highest subpopulation (2830 m) was separated from the others (Fig. 1, the middle of diagram), and the other two groups were further divided into two smaller groups, respectively. The result suggested that individuals of the same subpopulation had similar genetic variability, while the 5 subpopulations were separated from each other. These results were confirmed by the analysis of Gower's (1985) similarity between the individuals of the same subpopulation ($R = 0.59$, $P < 0.001$). In addition, an analysis of principal factors also showed three similar groups, with the highest subpopulation (individuals 21 ~ 25) being clearly separated from the others (Fig. 2). By Mantel test, we transformed the matrix of similarity into Jaccard's (1908) matrix of distance and analysed the correlation between the matrix of distance and the differences of altitude. The results also revealed a significant positive correlation between the genetic distance and altitude ($R = 0.64$, $P < 0.001$ and 999 permutations were used). Moreover, the analysis of paired matrix of distance between the subpopulations showed that genetic differentiation between the subpopulations

along altitudinal gradient was significant ($P < 0.05$) except for the subpopulations 2020 ~ 2200 and 2405 ~ 2575 ($P = 0.144$)(Table 2), because these two subpopulations were similar in genetic variability and were in an identical group in the above analysis (see Fig. 1 and Fig. 2).

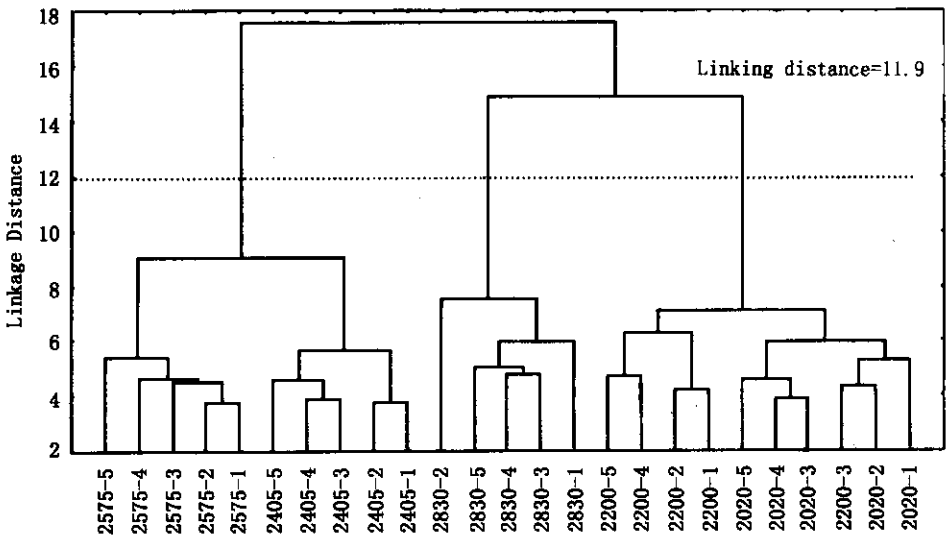

Fig.1　Tree Diagram for 25 Cases; Ward's method; Euclidean distance

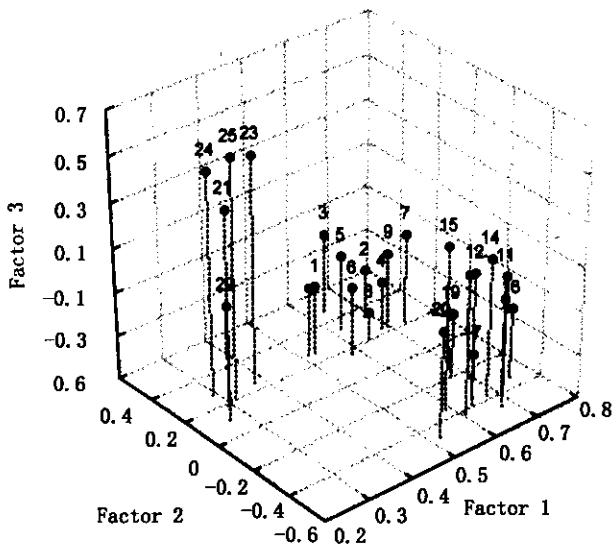

Fig.2　Factor Loadings, Factor 1; Unrotated; Principal factors(Centroid)

Table 2 Pairwise analysis of distance matrix of RAPD and significant level (P) measured by Mantel test.

pairs of subpopulations	P
2020-2200	ns
2020-2405	*
2020-2575	*
2020-2830	*
2200-2405	*
2200-2575	*
2200-2830	*
2405-2575	ns
2405-2830	*
2575-2830	*

Note: ns——no significant; *——$P < 0.05$

3 Discussion

Genetic differentiation for morphological and allozymic, as well as DNA characters in natural plant populations along a geographic distance is a common observation (Dawson *et al.*, 1993; Saghai Maroof *et al.*, 1990; Mitton *et al.*, 1980; Bergmann, 1978; Tigerstedt, 1974; Clegg & Allard, 1972). Traditional views assumed that there would be little genetic differentiation within continuous populations (Mayr, 1963), but, more recent observations illustrated that genetic differentiation does occur over short distances in many plants (Galen *et al.*, 1991; Bos *et al.*, 1986; Jain & Bradshaw, 1966; Grant & Hunter, 1962) as the result either of spatial variation in heterogeneous environments or of local gene flow (Brown, 1979; Endler, 1977; Levin an Kerster, 1974; Bradshaw, 1972). Higher levels of gene flow can counteract the action of selection by reducing genetic differentiation among populations (Jain & Bradshaw, 1966). Restricted gene flow may permit local adaptation (Slatkin, 1985; Dickinson & Antonovics, 1973; Jain & Bradshaw, 1966) and can foster genetic isolation by distance (Endler, 1977; Wright, 1951).

Despite the presence of few genetically identical RAPD genotypes within subpopulations, which indicates that *A. alpinum* is a highly outcrossing species with a corresponding high degree of genetic variation, a significant subpopulation differentiation within the transect was revealed using RAPD markers. A good agreement was obtained from the results of the different statistical methods used for analysing population structure in this study.

In our study sites, *A. alpinum* grows in a continuous population along the altitude gradient. However, genetic differentiation was not found to be random as a significant correlation was observed in this study, which suggests that the differences of altitude between subpopulation sites may result in the differences of phenological period for flowering and growth which restrict gene flow.

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摘要 应用 RAPD 技术,沿一个海拔梯度研究了阿尔卑斯山黄花茅自然居群的遗传变异和分化。实验表明,虽然在亚居群间有很少的亚居群独有遗传标记的存在,但通过 RAPD 资料的聚类分析、主因子分析以及相关分析观察到遗传分化沿海拔梯度发生,而且亚居群间的遗传分化和它们的海拔高度(地理距离)呈有意义的正相关。研究结果暗示,在阿尔卑斯山的高山-亚高山过渡区,亚居群间的海拔高度差别可能导致黄花茅开花和生长物候期的变化,而后者限制了亚居群间的基因流,从而引起居群内的遗传分化。

关键词 黄花茅; 遗传分化; RAPD; 海拔梯度